UPREGULATION OF MATRIX METALLOPROTEINASES – 2 AND -9 AND TYPE IV COLLAGEN DEGRADATION IN SKELETAL MUSCLE REPERFUSION INJURY

Denise Margaret Roach, MBBS, DDU, FRACS.

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The work described was performed within the Department of Surgery,
The University of Adelaide, The Queen Elizabeth Hospital, Adelaide, Australia

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Abstract

Aims and objectives: To determine the role of the matrix metalloproteinases, MMP-2 and MMP-9, in reperfusion injury following skeletal muscle ischaemia and to determine whether inhibition of MMPs by doxycycline protects against tissue damage.

Methods: Sprague Dawley rats were anaesthetised and a tourniquet applied above the greater trochanter to occlude blood flow. Sham-operated rats underwent four hours of ischaemia and were sacrificed after 0, 4, 24 or 72 hours. Four hours of unilateral or bilateral lower limb ischaemia was followed by reperfusion for 0, 4, 24 or 72 hours. Two groups of rats received 50 mg/kg or 200 mg/kg twice daily doxycycline for 7 days prior to bilateral ischaemia and 24 hours of reperfusion. Rats were euthanased and skeletal muscle from both limbs, pulmonary and renal tissues were harvested for wet/dry weight lung ratios, histopathological analysis, zymography, western blot analysis and immunohistochemical staining for type IV collagen.

Results: Histopathological analysis confirmed the validity of the animal model with significant tissue damage seen in ischaemic skeletal muscle and kidney. Upregulation of MMP-2 and MMP-9 was seen on zymography in the ischaemic leg and lung but not in the kidney. Western blot analysis with MMP-9 antibody confirmed the zymographic findings. Quantitative immunohistochemical analysis of levels of type IV collagen, showed degradation in reperfused muscle, lung and kidney. There was less upregulation of MMP-2 and MMP-9 in the skeletal muscle seen on zymography following pre-treatment with doxycycline. Doxycycline treated rats showed significant preservation of type IV collagen in skeletal muscle and partial protection from type IV collagen degradation in lung and kidney. The lung wet/dry weight ratios showed no statistical difference between sham-operated and ischaemic animals.

Conclusions: MMP-2 and MMP-9 are strongly upregulated in skeletal muscle ischaemia/reperfusion injury and are also upregulated in remote organs, leading to degradation of membranes. Inhibition of MMP activity may therefore be potentially therapeutically useful in reducing the severity of reperfusion injury.